***Original Research Article***

# Proximate analysis, lipid profile, microbiological and pigment characterization of *Chlorella dry powder* produced *in* a 20 L agitated photobioreactor

.


## ABSTRACT

**Aims:** The aim of this work was to investigate the massive production of *Chlorella* with food purposes. The second objective was the characterization of the dried product, in terms of proximal, microbiological, pigment and specific lipids analysis. **Place and Duration of Study:** This work was carried out mostly in the bioprocess laboratory, UPIBI-Instituto Politecnico Nacional in 2016. **Methodology:** The *Chorella* Powder was cultivated in a 20 L agitated tank. *Chlorella* was separated from the media, dried and characterized in terms of proximal analysis, lipid profile, microbiological and pigments. Results were compared with a commercial product. **Results:** It was feasible the production of *Chlorella* in a 20 L agitated reactor in outdoor conditions. The calculated value of $\mu$ was 0.093 $day^{-1}$. The *Chlorella* product presented similar characteristics to a commercial product maybe except in the protein content (the commercial *Chlorella* had a protein contain up to 40%and the home-made product, 21%). The microbiological characterization indicated that both home-made and commercial products are able to be used as human food or supplement. The analysis of pigments by TLC showed the presence of zeaxhantine, $\beta$-carotene, chlorophyll a, pheophorbide and pheophitine. The lipids of the home-made product were composed basically of $\alpha$-linoleic acid (23.4% in molar basis) and palmitic acid (17.5%), followed by linoleic (13.5%) and stearic acids (12%), while the commercial product lipids were composed mainly by $\alpha$–linoleic (26.9%) and palmitoleic acids (19.1%), followed by palmitic acid (18.9%). **Conclusion:** It was feasible the production of *Chlorella* in a 20 L agitated reactor in outdoor conditions. The home-made product is very similar to the comercial one. It can be used as nutraceutic, providing with proteins, minerals, antioxidant and healthy lipids to the consumer.


*Keywords:* agitated photobioreactor, *Chlorella*, food, lipids, pigments, proteins.

## 1. INTRODUCTION

Microalgae such as *Chlorella* can be massively produced using different systems including airlift loop bioreactors ALB (Ying et al 2013), bubble-column photobioreactors BCP (Loures et al, 2015) or agitated photobioreactor AP (Kumar et al, 2016) in both batch and chemiostate operation modes. Employed growth medium is in general a defined salts medium, but the use of different wastewaters from different sources has been also reported (Zhang and Hong, 2014).

There are different applications for *Chlorella* production. One of them is the use of the dry microalgae in order to extract the lipids produced and use them for biodiesel production (Mostafa et al, 2014; Zhang and Hong, 2014; Loures et al, 2015;Kumar et al, 2016). The

other biomass application is as human or animal supplement. (Seyfabadi et al,2011; Bishop and Zubeck, 2012; Kent et al,2015).

Nutraceuticals are food or part of food that not only supplement the diet, but also facilitate the prevention or treatment of a disease and/or disorder (Bishop and Zubeck, 2012). The current estimated global market size for nutraceutical products is 30-60 billion dollars. Microalgae are a diverse group of autotrophic organisms that have the ability to grow rapidly, efficiently use light energy, fix atmospheric $CO_2$, and produce more biomass per acre than plants. Microalgae that are currently employed as nutraceuticals are *Chlorella, Dunandiella, Haematococcus, Aphanizomenon* and *Spirulina.* This is due to their vitamins, K, Na, I, Se, Fe, Mn, Cu, P, Na, N, Mg, Co, Mo, S and Ca contents. These algae are also producers of essential aminoacids and specific lipids, such as omega 6 and decosahexanoic acid. (Bishop and Zubeck, 2012).

Regarding the use of *Chlorella* as nutraceutic, it has been reported that this microalgae posses diverse antitumor, antioxidant, anti-inflammatory and antimicrobial activities, *Chlorella* is able to decrease blood pressure, lower cholesterol levels, accelerate wound healing and enhance the immune system (Bishop and Zubeck, 2012).The United States, Japan, China. Taiwan and Indonesia produce over 2,500 tons of dried *Chlorella* each year (Gupta and Mukerji, 2001). It has been published that *Chlorella* dry powders contain about 40, 16, 25 and 6% of proteins, lipids, carbohydrates and ashes. Besides, *Chlorella* contains chlorophylls, carotenoids, astaxantine and β-carotene (Kent et all, 2015). Finally, Bishop and Zubeck (2012) found vitamins $B_1$, $B_3$, $B_5$, B6, $B_9$ and $B_{12}$ , in *Chlorella* dry product besides chlorophyll, β−carotene and Mg.

The aim of this work was to show how feasible is the massive production (20L) of microalgae with food purposes, using a strain of *Chlorella*. The second objective is to determine some characteristics of the dried product, such as proximal analysis, microbiological characteristics pigment and lipids analysis.

## 2. MATERIAL AND METHODS

### 2.1 *Chlorella* strain

The employed *Chlorella* sp. strain was isolated from a consortium composed by *Chlorella vulgaris, Scenedesmus* sp. and some diatomeas employed in previous works (Torres et al, 2016).

### 2.2 Cultivation in the 20 L fotobioreactor

The cultivation was performed in a 20 L agitated fotobioreactor, under ambient conditions for 14 days in the BBM medium. Cultures were aerated 12 hours a day by a perforated ring placed at the base of the bioreactor. Biomass development was followed by reading optical density. Previously a calibration curve DO *vs* dry weight was prepared. The variables were measured throughout the process once a day (about 10 am) was biomass; pH, conductivity, outside temperature, and the photon irradiance μmols photons $m^{-2}s^{-1}$ were measured. Harvest was carried out by sedimentation. The supernatant was repeatedly retired from the reactor until a small amount of sludge was obtained. Total amount of microalgae were centrifuged. The material thus produced was dried at environment conditions, and it was ground and stored for later proximate analysis. Commercial *Chlorella* product (Vidanat, Mexico) was characterized with comparison purposes.

### 2.3 Proximate composition

The proximate composition of products was determined according to the AOAC (1997) International methods, namely nitrogen (954.01); fat (920.39); ash (923.03); crude fiber (962.09); humidity (925.09); and total carbohydrates calculated by differences.

### 2.4 Microbiological characterization

The two dried products were analyzed in terms of fungi, yeast, total and faecal coliforms, *Salmonella* and *Shigella* contents, in accord to a Mexican norm (SSA,1994).

### 2.5 Pigments analysis

The pigments present in the dry product (home-made and commercial products) were analyzed as follows: 0.5 g of product was mixed with acetone for analysis and vortexed. Then the mud was put in a ceramic mortar and milled successively, adding as much as acetone as necessary (about 5 times).From this mud, take only 0.1 g and suspend in 1 mL of acetone in an eppendorf tube. Close and maintain from this point on in ice and covered with aluminum foil to prevent light damage. The eppendorf tube is centrifuged at 16,000 and low temperature ($4^{o}$C) during 10 min. Take out the pellet repeat centrifugation step. Reserve the acetone containing the pigments. Prepare about 100 mL of a mixture of petroleum ether (70%) and acetone (30%) in a large precipitation flask was the TLC can be collocated. The TLC layers employed were 5x20, 0.2 mm aluminum oxide N (Macherey-Nagel Co, Germany). The layer is charged in the bottom side with 10 mL of every pigment solution, very slowly and carefully trying to produce a small spot over the layer. The mixture of solvents is inside the precipitates flask and is in such amount than when the layer is put inside the flask, the pigment spots do not touch the solvent. The layer is collocated in an inclinated way and the flask is covered with aluminum foil in order to prevent the solvent evaporation as much as possible. The solvent starts to run through the layer and the pigments start to separate. The process is allowed until the solvent is 1-2 mm before the end of the layer. The layer is took out from the flasks and allowed to dry completely. Using a small spatula, every pigment from the layer is scratched and suspended in1 mL of acetone (80%) and water (20%) in an eppendorf tube, and centrifuged. This process is repeated if still some aluminum oxide particles are evidently floating on the solvent. Every pigment solution is read in a UV/Vis spectrophotometer (Perkin-Elmer Lamda 25). The profiles of abundance vs. wave length are stored and compared against those profiles previously reported in Jeffrey et al (1997).

### 2.6 Lipids isolation and quantification

Lipids were extracted following the method of Bligh and Dyer (1959) and then, were saponified and methylated by the method of Slover and Lanza (1979) Fatty acid profiles were obtained using a FOCUS gas-chromatography instrument (Thermo Electron Corporation, Les Ulis, France) equipped with a flame-ionization detector.

## 3. RESULTS AND DISCUSSION

### 3.1 Growth of *Chlorella* in the photobioreactor

Table 1 show the parameters measured during the growth of *Chlorella* in the 20 L photobioreactor. *Chlorella* strain started to grow immediately (no lag phase was observed). At day $14^{th}$ reached the maximum biomass of about 1.4 g/L. This value is higher to that reported by Mostafa et al (2012) for *Chlorella vulgaris* in municipal wastewaters at flask level at pH= 8.11 (1.052 g/L). The calculated value of $\mu$ is 0.093 $day^{-1}$. This value is lower to that reported for Zhang and Hong (2014) for *Chlorella sp.* growing in different wastewaters in

sterile conditions ($\mu$= 0.39-0.47 days$^{-1}$) but similar to the growing rate, when *Chlorella* grew in non-sterile conditions ($\mu$= 0.07-0.19 days$^{-1}$). On the other hand, Chiu et al (2009) grew a strain of *Chlorella* in three different air-lift photobioreactors, reaching final biomass concentrations of 2.3-3.4 g/L and m values of 0.15-0.25 days$^{-1}$, when *Chlorella* grew in artificial seawater for 11 days.

**Table 1.** Parameters measured at 10 am during the 14 days of *Chlorella* culture.

| Day | Absorbancy (750nm) | Conductivity (ms) | pH | Irradiation (µmol/m$^2$s) | Tempera-ture °c | Sky |
|---|---|---|---|---|---|---|
| 1 | 0.066 | 26.3 | 9.77 | 1613 | 20 | Clear |
| 2 | 0.101 | 25.3 | 9.89 | 1400 | 22 | |
| 3 | 0.139 | 26.0 | 9.93 | 1782 | 21 | |
| 4 | 0.168 | 25.2 | 9.91 | 1883 | 20 | |
| 5 | 0.235 | 25.6 | 10.12 | 1710 | 21 | |
| 6 | 0.368 | 24.5 | 10.14 | 1600 | 22 | |
| 7 | 0.458 | 25.3 | 10.19 | 1660 | 20 | |
| 8 | 0.569 | 25.5 | 10.22 | 1500 | 21 | |
| 9 | 0.629 | 26.4 | 10.26 | 1340 | 22 | |
| 10 | 0.701 | 25.3 | 10.31 | 1450 | 20 | |
| 11 | 0.856 | 24.9 | 10.38 | 1420 | 21 | |
| 12 | 0.96 | 26.6 | 10.40 | 547 | 19 | Cloudy |
| 13 | 1.152 | 25.6 | 10.42 | 1250 | 20 | Clear |
| 14 | 1.256 | 25.4 | 10.45 | 780 | 19 | Cloudy |

As showed in the mentioned table, conductivity (as a measure of salinity) did not change very markedly during the process. pH values started in 9.8 and tend to increase up to the final of the culture, reaching a final value of 10.45. The daily irradiance received by the bioreactor was characterized as the rest of the parameters once a day (10 am). Irradiances fluctuated between 547 and 1,880 µmol photons/m$^2$s. Days twelve and fourteen were very cloudy and irradiation was very low (547 and 780 µmol/m$^2$s at 10 am, respectively). The rest of the days, sky was very clear and irradiation was higher than 1,250 µmol/m$^2$s.

At the end of the process, the amount of biomass in the reactor was 1.4 g/L, meaning a biomass productivity $P_X$ of 100 mg/L.day. Regarding the lipids, the final concentration was of 19 mg/L, giving a lipid productivity $P_L$ of 1,35 mg/L.day. Regarding these values, Loures et al (2015) reported the growth of *Chlorella minutisima* in bubble column photobioreactors of 50L, using the Guillard f/2 medium. They reached final biomass values of 280-320 mg/L and biomass productivities between 40 and 46 mg/L.day (the half $P_X$ that the one reached in this work). Regarding the lipids, they reached final dry product concentrations between 31.5 and 34.3%, giving lipid productivities between 1,37 and 1.45 mg/L.day, very similar values to those obtained in this work.

Zhang and Hong (2014) reported that *Chlorella*, growing in different wastewaters reached biomass productivities $P_X$ between 100 and 460 mg/L.day, while lipids showed productivities $P_L$ between 0.8 and 4.25 mg/L.day.

### 3.2 Characteristics of the dry powder

The final product (around 25 g) was characterized in terms of the proximal analysis, microbiological parameters and pigments profile. The commercial product *Chlorella* Vidanat (Mexico) was also characterized with comparison purposes. Table 2 show the proximal

analysis for the home-made and commercial *Chlorella* samples, as well as the results for a batch of dry *Spirulina* produced by our research group and published elsewhere (Torres et al, 2016).

It is noteworthy that the commercial and home-made *Chlorella* products are not so similar among themselves. While the carbohydrate content of commercial *Spirulina* is 6.52%, our home made product present 45.7%. Regarding the proteins level, the commercial product reached a concentration of about 38%, while the commercial sample showed a 21% content. Regarding lipids, the commercial product showed a concentration of 3.9% and our product 1.4%. Raw fiber showed contains were of 38.7 and 14.5 for the commercial and home-made product. Ashes showed values of 4.4 and 11.6% in the same order. Finally, humidity was quite similar with a content of 7.6% for the commercial *Chlorella* and 5.9 for our home-made product.

**Table 2**. Proximate analysis for the *Chlorella* dry products. Home-made *Spirullina* and commercial *Spirulina* are included with comparison purposes.

| Sample | Carbohydrates (%) | Proteins (%) | Lipids (%) | Raw fiber (%) | Ashes (%) | Humidity (%) |
|---|---|---|---|---|---|---|
| Home-made *chlorella* | 45.75 | 20.84 | 1.39 | 14.54 | 11.60 | 5.88 |
| *Chlorella* vidanat | 6.52 | 38.85 | 3.87 | 38.73 | 4.40 | 7.66 |
| Home-made *spirulina** | 9.16 | 60.74 | 10.24 | 3.77 | 9.65 | 6.41 |

**From Torres et al.*[10]

The comparison of the *Chlorella* products against the *Spirulina* dry powders, showed big differences. First, the protein levels in the *Spirulina* samples showed values up to 60%. Lipid contents were also higher in *Spirulina* than in *Chlorella* samples (8-19%). Raw fiber and carbohydrates values were also quite different. *Spirulina* dry product has been employed as nutraceutic for a long time. *Chlorella* has been commercialized also, in a lower scale. Even mixtures of *Spirulina/Chlorella* products have been offered by some international companies. Zeyfabadi et al (2011) reported that *Chlorella* dry product contained between 33 and 46% of protein, depending on the irradiance (37.5 to 100 μmol photons $m^2$/s), and the employed photoperiod (8:16, 12:12, and 16:8 light/dark hours). Protein content seems to be too high (higher than that reported for *Spirulina* products, about 60%). Bishop and Zubeck (2012) reviewed *Chlorella* dry products characteristics, founding protein, fat, and carbohydrates levels of about 64.5, 10, 15 and 5%. More conservative data reported by Kent et al (2015), establish ash, carbohydrates, lipids and protein contents of 5.7, 24.9, 16.1 and 40%, respectively.

The microbiological analysis of the samples was submitted to the analysis proposed by the Mexican norm and results are showed at Table 3. It is clear that both commercial and home-made *Chlorella* products are within the levels proposed for a food product. Mesophylic bacteria were in a concentration < 10 col $g^{-1}$, as well as yeasts, molds and non pathogenic coliforms. *Salmonella, Shigella* and enteropathogenic *E. coli* were absent from the samples in both products. That means that both dry products are safe for human consume.
The pigment analysis of the *Chlorella* samples showed the presence of various pigments and some degradation products (See Fig 1).

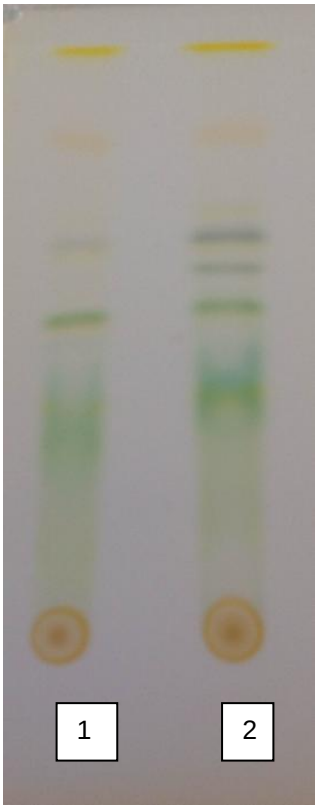


**Figure 1**. TLC for the commercial (1) and home-made (2) *Chlorella.*

**Table 3.** Microbiological analysis for the dry products.

| Specification | Maximum for food products | *Home made Chlorella* | *Chlorella* vidanat |
|---|---|---|---|
| Mesophylic aerobic bacteria | 50,000 col $g^{-1}$ | < 10 | < 10 |
| Yeasts | 10 col $g^{-1}$ | < 10 | < 10 |
| Molds | 10 col $g^{-1}$ | < 10 | < 10 |
| Non pathogenic coliforms | Negative | < 10 | < 10 |
| *Salmonella* | Negative | Negative | Negative |
| *Shigella* | Negative | Negative | Negative |
| Enteropathogenic *E. coli* | Negative | Negative | Negative |

The analysis of the bands obtained trough TLC gave as result, the identification of a group of pigments and their derivatives. Most of the products were common for both the home-made and commercial *Chlorella* samples. As observed in Table 4, both *Chlorella* showed nearly the same pigments and derivatives. Both samples showed the presence of Zeaxanthine (code 22A), a simple carotenol very related with Lutein and , β-carotene (code A34). All carotenoids are yellow to red isoprenoid polyene pigments, widely distributed in nature (Liaaen–Jensen and Egeland, 1999).

**Table 4**. Pigment contents in the home-made and commercial samples of *Chlorella.*

| Chlorella Sample | Band code | Pigment(s) | Remarks |
|---|---|---|---|
| Home-made | 22A | Zeaxanthine | Carotenol |
| | 22B | Pheophytine-like | Chlorophyll degradation product |
| | 22C | Pheophytine a | |
| | 22G | Chlorophyll b | Chlorophyll |
| | 22K | Pheophorbide a | Chlorophyll degradation product |
| | 22L | Pheophytine a | |
| | 34E | β-carotene | Carotenol |
| Commercial Product | 22A | Zeaxanthine | |
| | 21B | Pheophytine a | Chlorophyll degradation product |
| | 22B | Pheophytine-like | |
| | 22C | Pheophytine a | |
| | 22G | Chlorophyll b | Chlorophyll |
| | 22K | Pheophorbide a | Chlorophyll degradation product |
| | 22L | Pheophytine a | |
| | 34E | β-carotene | Carotenol |

Zeaxhantine has been found in in Cyanophyta, Prochlorophyta, Rhodophyta and Chlorophyaceae division/class as a major (>10%) or minor (1-10%) pigment (Jefferey et al (1997), and even in Eustigmatophyta as trace pigment (<1%). Also, in both samples was found Chlorophyll a. This is a very common pigment, found in Cianophyte, Rodophyta, Cryptophyta, Chlorophyaceae, Prasinophyaceae, Euglenophyta, Bacillariophyta, Dinophyta, Primnesiophyceae and Chrysophyaceae division/class. Chlorophylls are green pigments that form photosynthetic complexes with carotenoids. All chlorophylls are a group of Mg coordination complexes of cyclic tetrapyrroles. Pheophitine a and pheophorbide a were found in both *Chlorella* samples. These molecules are degradation products of Chlorophylls. They have been used as markers for intertidal microbenthos grazing (Cartaxana et al (2003). Kent et al, 2015 reported that *Chlorella* contain about 8.60 mg/g of chlorophyll summation, 1.17 mg/g of carotenoids summation, 0.08 mg/g of astaxantine and 0.19 mg/g of β-carotene. On the other hand, Seyfabadi et al (2011) reported chlorophyll productions for *Chlorella* vulgaris between 7.4 and 13.1 mg/L, depending on the irradiance and photoperiod values employed during the microalgae culture. Regarding β-carotene, these authors mentioned that final biomass contained from 0.02 to 0.07 pg/cell. Finally, Bishop and Zubeck (2102) mentioned that the *Chlorella* product contained 0.086 and 5% of β-carotene and chlorophyll, respectively.

The microalgae pigment contents are of particular interest, since these products show high added values in the market, with prices of 0.1-10 USA$/kg for chlorophylls (Gonzalez-Delgado et al, 2013) , 882 USA$/kg for astaxantine (Li et al, 2011), and 300-3,000 USA$/kg for β-carotene (Hannon et al, 2010). The estimated global markets for pigments are quite huge, i.e., 20, 235 and 275 million USA$/year for zeaxhantine, astaxantine and β-carotene, respectively, as examples (Borowitzka, 2013).

Regarding the analysis of lipids, Table 5 show the profile of fatty acids found in both the commercial and home-made samples. As shown, both microalgae samples are rather similar regarding the presence of different lipids, but not regarding the amounts of every fatty acid.

**5.** Total lipid fatty acid composition of the home-made and commercial *Chlorella* products (% molar).

| FAME | SAT FAME | | | MUFA | SAT FAME | PUFA | | | SAT FAME | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | C12:0 | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | C20:0 | C22:0 | Total |
| Common names | Lauric | Myristic | Palmi-tic | Palmi-toleic | Stea-ric | Oleic | Linole-ic | α-linoleic | Arach-idic | Behe-ntic | - |
| Home-made *Chlorella* | Nm | 0.45 | 17.49 | 8.33 | 12.04 | 4.67 | 13.48 | 23.38 | NM | NM | 88.41 |
| Commer-cial product | Nm | 0.23 | 18.88 | 19.15 | 7.64 | 13.47 | 9.11 | 26.91 | NM | NM | 88.97 |
| *Chlorella sorokinia-na** | 2.37 | 0.27 | 19.72 | 11.76 | 1.35 | 29.85 | 31.8 | NR | 0.08 | 0.03 | 97.23 |

*From Kumar et al.[3]. NR- Not reported. NM not measured. SAT FAME-saturated fatty acids, MUFA- monounsaturated fatty acids, PUFA-polyinsaturated fatty acids.

The lipids of the home-made product were composed basically of α-linoleic acid C18:3 (23.4% in molar basis) and palmitic acid C16:0 (17.5%), followed by linoleic acid C18.2 (13.5%) and stearic acid C18.0 (12%). It can be mentioned that a peak between C16:1 and C18:0 with a retention time of 10.81 was observed. This peak corresponds to an 8.07% and could be presumably the C16:2. Because of the lack of standard, it cannot be confirmed. Total lipids do not sum 100% because of the presence of small peaks with molar percentages less than 1%.

On the other side, the commercial product lipids were composed mainly by α-linoleic acid C18:3 (26.9%) and palmitoleic acid C16:1 (19.1%), followed by palmitic acid C16:0 (18.9%). Again, a peak between C16:1 and C18:0 with a retention time of 10.83 was observed. This peak corresponds to a 3.31% and could be presumably the C16:2. Because of the lack of standard, it cannot be confirmed. Total lipids do not sum 100% because of the presence of small peaks with molar percentages less than 1%.

Regarding other works with *Chlorella* strains, Kumar et al (2016) reported the fatty acid composition for *Chlorella sorokiniana* under different modes (fed batch and various dilution rates D values in continuous cultures). Table 5 show the fatty acid content corresponding to *Chlorella* grown at feed batch conditions. Note that the lipids produced were mainly linoleic acid C18:2 (31.8%) and oleic acid C18:1 (29.85), followed by palmitic acid C16:0 (19.7%). Besides, these authors reported the presence of small quantities of lauric acid C12:0 (2.4%), arachidic acid C20:0 (0.08%) and behenic acid C22:0 (0.03%).

From the point of view of microalgae as human food/nutraceutic, it is important to distinguish the amount and type of FAMEs present in the *Chlorella* products. The home made product had the highest SAT FAME level, followed by *Chlorella sorkiniana* and the commercial product. The effect of the saturated fatty acids is still controversial. Some authors reported that the ingestion of SAT FAME, except stearic acid is prejudicial for health. Nevertheless, the SAT FAME levels are lower than those reported for other foods such as animal meat. Romero et al (2013) reported SAT FAME percentages of 39, 45, 39 and 43% of the total lipids for *salamin, chorizo, morcilla* and *chorizo ahumado* (all meat sausages from Argentina).

Regarding the MUFAs, The highest value was for the commercial product, followed by the *Chlorella sorkiniana* sample and our home –made product at the end. It has been reported that diets with healthy amounts of monosaturated fats have health benefits including: 1) decrease risk for breast cancer, 2) reduced cholesterol levels, c) lower risk for heart disease and stroke, d) weight loss, e) less severe pain and stiffness for suffers of rheumatoid arthritis and f) reduced belly fat.

On the other hand, the PUFAs levels for the *Chlorella samples* were higher for the commercial product, followed by the home-made and the *Chlorella sorkiniana* samples, There is substantial evidence that PUFAs induce significant beneficial cardiovascular effects (Ander et al, 2003). Other interesting index is the PUFA/SAT FAME, where the commercial sample had the higher value, followed by the home made and the *Chlorella sorkiniana* products. The higher the index, the better the product. Regarding the PUFA/SAT FAME-stearic acid index, Best value was for the home-made product, followed by the commercial product and *Chlorella sorkiniana* sample, at the end. Finally, the index of MUFA+PUFA/SAT FAME–stearic acid had the best value for the commercial product, followed by the home-made product and the *Chlorella sorkiniana* sample, and the last two were almost identical.

## 4. CONCLUSION

The production of a home-made product through a strain of *Chlorella* growing in a 21 L agitated tank at outdoor conditions was presented. Some production characteristics such as the irradiance, temperature, pH, conductivities (as a measure of salinity) and other parameters were presented and discussed. The final product was dried and milled, and then, characterized extensively.

The product presented similar characteristics to a commercial *Chlorella* product maybe except in regards to the protein content. The protein content was about 21%, while the commercial *Chlorella* had a protein contain up to 39%. The microbiological characterization indicated that both home-made and commercial products are able to be used as human food or supplement. The analysis of pigments by TLC showed the presence of zeaxhantine, $\beta$-carotene and chlorophyll a, though no quantification was carried out. Other products such as pheophorbide and pheophitine a, were also identificated.

Regarding the analysis of lipids, both microalgae samples resulted rather similar regarding the presence of different lipids, but not regarding the amounts of every fatty acid. The lipids of the home-made product were composed basically of $\alpha$-linoleic acid C18:3 (23.4% in molar basis) and palmitic acid C16:0 (17.5%), followed by linoleic acidC18.2 (13.5%) and stearic acid C18.0 (12%), while the commercial product lipids were composed mainly by $\alpha$-linoleic acid C18:3 (26.9%) and palmitoleic acid C16:1 (19.1%), followed by palmitic acid C16:0 (18.9%).

More work on the optimization of the photobioreactor operation must be carried out, i.e. the effect of the irradiation and temperature over the proteins and pigments concentrations. It can be said that we have produced a dry *Chlorella* product susceptible of being used as nutraceutic or food supplement.

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